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Orexin and modafinil

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14. ABSTRACT Metabolic diseases are known to be tightly linked to arousal-sleep cycles and impact cognitive function. Importantly, the armed forces represent a population at significant risk for increased stress and disrupted arousal-sleep cycles. Because the incidence of metabolic disease and obesity is increasing, even in these physically fit individuals, understanding the interactions between these systems is highly significant. Further, some anti-fatigue pharmacologies (e.g.,modafinil) are <i>already used</i> in military settings, though their long-term effects on metabolism or central nervous system function are not well-understood. We have completed Year 3 of the proposed funding period to assess the physiological and behavioral effects of this pharmacology on rat subjects and identify potential molecular mechanisms activated by nutrients. We have begun additional studies to elucidate the specific nutrients that confer these effects with an eye toward specifically identifying the source of possible beneficial consequences for stress-reduction and cognition. Additionally, we have recently observed chronic stress can differentially regulate expression of the orexin system in CNS circuits critical to complex behavior and memory processes. Finally, we have made further progress on identifying the molecular and cellular mechanisms of these systems.						
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## Introduction:

The incidence of obesity is escalating to epidemic proportions in all segments of society. Even among the military, with much higher levels of fitness than civilian populations, are experiencing a rapid increase in obesity and metabolic disease. Recent research has suggested an important connection between arousal/stress physiology and metabolism. One important basis for this connection may be the brain orexin-A system, which is also the principal target of the anti-fatigue drug modafinil. However, the specific molecular machinery remains unidentified, as do the behavioral effects of manipulating this system. **Objective/Hypotheses:** We have hypothesized that modafinil and other anti-fatigue drugs may act by modulating metabolic pathways in the central nervous system. We also hypothesize that chronic stress and disruption of arousal-sleep system leads to impaired metabolic function and increased susceptibility to obesity. Finally, we hypothesized that central metabolic pathways can be activated by foods and nutritional interventions, in lieu of pharmacological manipulation, with less risk of long-term metabolic complications. To assess these hypotheses, we are continuing several studies in rat subjects. During the third year of funding we have also pursued a suspected underlying metabolic pathway that might mediate the effects of nutrients on energy regulation as well as behavior. The mammalian target of Rapamycin (mTOR) kinase is a key regulator of several cellular functions, including cell growth and differentiation.

## Body:

During the third year of funding, we have made significant progress toward the stated aims and objectives. All proposed studies for Year 3 have been completed and we detail the results and conclusions here. For each study, we list the statement of work task, specific objectives, methods employed, and results obtained. Figures referenced are presented in an appendix at the end of the report. We note that there is overlap between Year 2 and Year 3 progress as the proposed tasks overlap these timeframes.

## Project 1 Year 3 Tasks:

Task 2: Identify metabolic pathways involved in modafinil action (19-36 months)

- Identify key nutrient sensitive molecules in orexin producing neurons.
- Determine whether nutrient sensitive pathways alter orexin neuronal activity.

## Experiment Series 2.1 Methods and Results

Because hypothalamic mTORC1 signaling has been implicated as a target of leptin in the regulation of energy balance, we investigated its role in obesity-induced leptin resistance. In contrast to rats maintained on a low-fat (LF) diet for 3 weeks, rats maintained on a HF-diet had no anorexic response to icv leptin (Figure 1). Western blot analysis revealed that leptin was unable to modulate hypothalamic mTORC1 signaling in the HF group, whereas it significantly induced phosphorylation of both S6 Kinase 1 (S6K1) and S6 ribosomal protein (S6) in the LF group. Similar to leptin, the cytokine ciliary neurotrophic factor (CNTF) induces hypophagia and increases STAT3 phosphorylation. However, CNTF and its analogue CNTF $\alpha$ 15 activate leptin-like pathways in the hypothalamus even in leptin-resistant states, including diet-induced obesity. Icv CNTF $\alpha$ 15 decreased 24-h food intake and body weight in rats on HF or LF diet and increased the

phosphorylation of hypothalamic S6K1 and S6 in a comparable way on both diets. Importantly, mice lacking the expression of S6K1 (S6K1<sup>-/-</sup>) did not respond to the anorectic action of either leptin or CNTFAx15, implying a crucial role for S6K1 in modulating the actions of these two cytokines.

Finally, exposure to HF diet decreased mTORC1 signaling within the hypothalamus (Figure 1) and increased mTOR signaling in hippocampus (Figure 2). Overall, these findings strongly point to the possibility that reduced hypothalamic mTORC1 signaling contributes to the development of hyperphagia, weight gain and leptin resistance during diet-induced obesity.

## **Experiment Series 2.2 Methods and Results**

In a separate set of studies, we have assessed the effects of food presentation on activation of the orexin system as well as context-based expectations of palatable foods. Briefly, rats were exposed to a novel context where they either received a palatable HF diet, no HF diet, *or in which they expected HF diet to be delivered*. They were then sacrificed by perfusion for c-fos immunohistochemistry in hypothalamus and cortical circuits. While the analyses are still underway, we have thus far observed that 1) palatable foods increase orexin neuron activation to a greater extent than do non palatable foods and 2) *even the expectation of a palatable food* increases activation of orexin expressing cells (Figure 3). Finally, we are in the process of a CNS-wide extensive quantification of regions that express fos under these conditions. We have thus far observed food and expectation-induced neuronal activation in the PFC, PVT, hypothalamus, and VTA (Figure 4). Importantly, many of the cells in these regions that express fos also express receptors for orexin. A manuscript describing portions of these data is currently in preparation.

## **Project 2 Year 3 Tasks:**

Task 2:

- Identify and assess the effects of chronic delivery of secondary pharmacological targets from Project 1 (months 17-36).

Task 3: Assess dietary interventions (months 13-36).

- Identify any key beneficial effects of dietary activation on arousal, memory systems, stress and behavior (months 24-30).
- Compare dietary administration and activation to pharmacological interventions (months 30-36).
- Assess dietary consequences on cognitive performance and behavior (months 30-40).

## **Experiment Series 2.1 Secondary targets: Methods and Results**

We have observed that chronic inhibition of the mTOR pathway attenuated reference, but not working memory (Figures 5 & 6). Briefly, rats were exposed to a pharmacological mTOR inhibitor (RAD, a rapamycin inhibitor) and trained in a spatial radial arm maze task. Inhibition of mTOR signaling blocked the formation of long-term memories, but had no affect on acute behavioral responses. The statistical significance of the data was analyzed by 1-way between-subjects ANOVA and Tukey's HSD post-hoc tests. Asterisks indicate statistically significant differences from vehicle treated rats.

## **Experiment Series 3.1 Dietary effects on behavior and cognition: Methods and Results**

In collaboration with our colleague Terry L. Davidson, we have also confirmed that diets high in fatty acids exert deleterious effects on cognition. Briefly, rats were maintained on either a high-fat (40% fat by kcal) diet or standard low-fat chow. They then underwent a reversal learning paradigm in which they first leaned that on CS (light or tone) predicted the delivery of sucrose pellets and another CS (again, light or tone) meant no sucrose would be delivered. After this training, the conditions were reversed, such that the CS previously paired with sucrose was no longer followed by sucrose pellets. Control rats acquire this “reversal learning” phase without difficulty. Rats with damage to the hippocampus, however, exhibit deficits. Therefore we predicted that the HF diet would attenuate the “reversal” or this task. Indeed this was the outcome as demonstrated by Figure 7. In order to assess whether the chronic HF affected downstream targets of mTOR signaling, we performed western blots on phosphorylated S6. As depicted in Figure 8, we observed no differences in the levels of hippocampal pS6 protein. This was confirmed by immunohistochemistry for pS6 in the hippocampus (e.g., Figure 9). While disappointing, these stat are important in that they suggest a potential target lies upstream of pS6 and we are currently assaying for pS6K as well as other markers of mTOR signaling.

### **Experiment Series 3.2 Effects of stress and nutrients on orexin signaling: Methods and Results**

In another series of experiments we have begun to assess the effects of stress on orexin signaling. Briefly, rats were first exposed to a 3-week chronic social stress, the visible burrow system (VBS). In the VBS, male rats develop a dominance hierarchy with some rats becoming “dominant” and some becoming “submissive.” Both DOM and SUB rats exhibited altered HPA axis function relative to home-cage controls as has been previously published. Also consistent with previous reports, we found that DOM rats spent a greater amount of time in the open-arms of an elevated plus maze (Figure 10, left panel). However, we also observed that DOM rats exhibited significantly increased motivation to obtain a palatable food (Figure 10, right panel). Further, we observed that DOM rats have significantly increased expression orexin mRNA and also orexin-1 receptor in the pre-frontal cortex (Figure 11). These novel findings have recently been accepted for publication in *Neuroscience*.

### **Experiment Series 3.2 Comparison of pharmacological and dietary interventions: Methods and Preliminary Results**

We are in the process of completing analyses from rats that have been maintained on several different diets (i.e., a 40% fat diet, low or high-protein diets, and standard lab chow). We are assaying brains from these rats for expression of orexin, orexin-receptor and genes related to the mTOR signaling pathways. Importantly, we are in the process of comparing body weight and behavioral activity responses of these rats to rats treated chronically with the orexin-receptor antagonist or rapamycin. The analyses are expected to be complete within the next 2-3 months.

## **Key Research Accomplishments:**

- Dietary nutrients and the amino acid leucine specifically acutely activate the mTOR pathways in the hypothalamus
- Context and memory dependent expectation of nutrients and palatable food activates lateral hypothalamic orexin neurons.
- Context and memory dependent expectation of nutrients and palatable food also activates a network of cortical circuits critical for memory and cognition.
- Pharmacological manipulation of the orexin system alters non-homeostatic ingestive behaviors.
- Chronic stress up-regulates expression of orexin and orexin-receptor mRNA.
- Orexin activation may mediate some of the behavioral and cognitive responses following chronic stress exposure.

## **Reportable Outcomes:**

### **Manuscripts**

1. Davis JF, Krause EG, Melhorn SJ, Sakai RR, Benoit SC (in press). Dominant rats display increased risk taking and augmented operant responding. *Neuroscience*.
2. Choi DL, Davis JF, Sakai RR, Benoit SC (in prep). Context-dependent activation of orexin neurons in food intake and stress.

### **Published abstracts**

3. Tracy AL, Krueger DA, Clegg DJ, Seeley RJ, Benoit SC (2008). The role of mammalian target of rapamycin (mTOR) in nutrient-mediated long-term memory processes. Society for Neuroscience.
4. Choi DJ, Davis JF, Benoit SC (2008). Orexin-1 receptor blockade attenuates non-homeostatic food intake. Society for Neuroscience.
5. Choi DL, Davis JF, Benoit SC (2009). Context-dependent expectation of food activates the orexin system. Society for the Study of Ingestive Behavior, to appear in *Appetite*.

### **Meeting presentations**

6. Choi DL, Davis JF, Schurdak JD, Clegg DJ, Benoit SC (2008). Role for orexin-A signaling on agouti-related peptide-induced hyperphagia. Cincinnati Neurofest.

**Conclusions:**

We have concluded that mTOR likely plays an important role in cognitive behaviors and long-term memory formation. Further, we have concluded that access to palatable foods increased orexin activation as well as activation of orexin-target neurons. We are in the process of assessing whether orexin directly activates mTOR signaling or whether these are parallel cellular events. Important, we have also concluded that orexin plays a role in the response to chronic social stress and may mediate the effects of chronic stress on ingestive behaviors. That is, we are beginning to understand that orexin and the cell-signaling molecule, mTOR play important roles in these cognitive and behavioral outcomes. Both can be manipulated by nutrients, both are responsive to stress and both may prove useful targets for pharmacological or nutrient-related treatments for improving cognitive performance in the face of stress and metabolic challenges.

**References:**

n/a

**Appendices:**

n/a

**Supporting Data:**

See next pages.

Figure 1.

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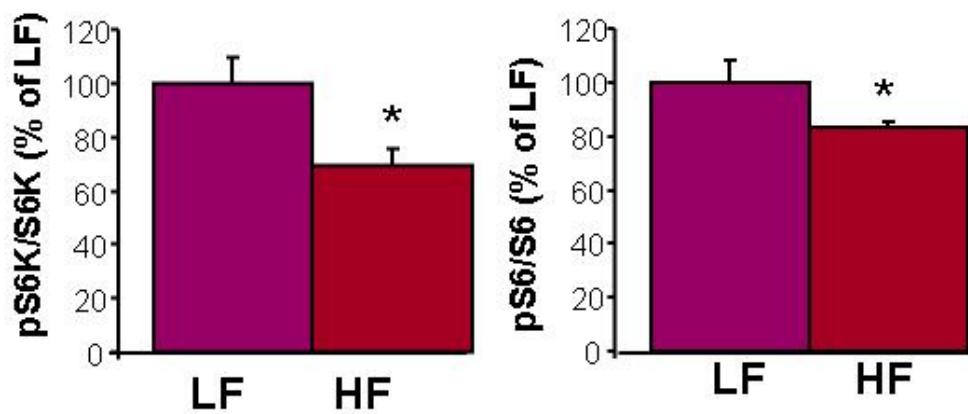
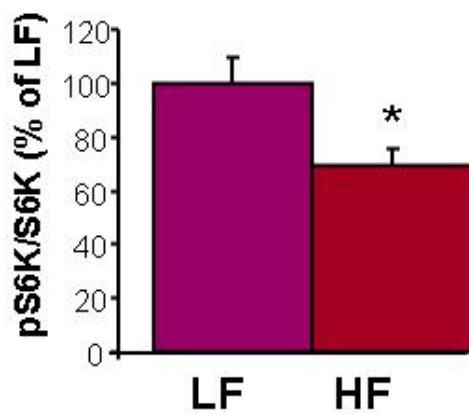
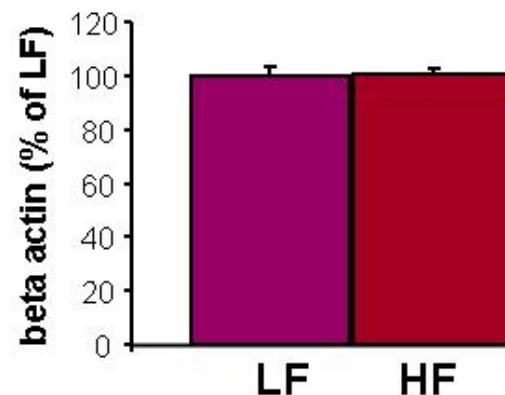
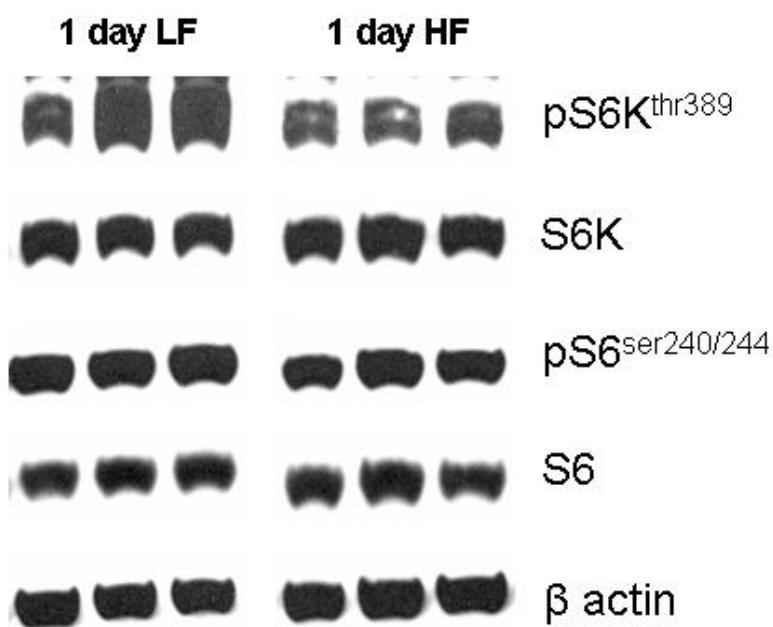


Figure 2.

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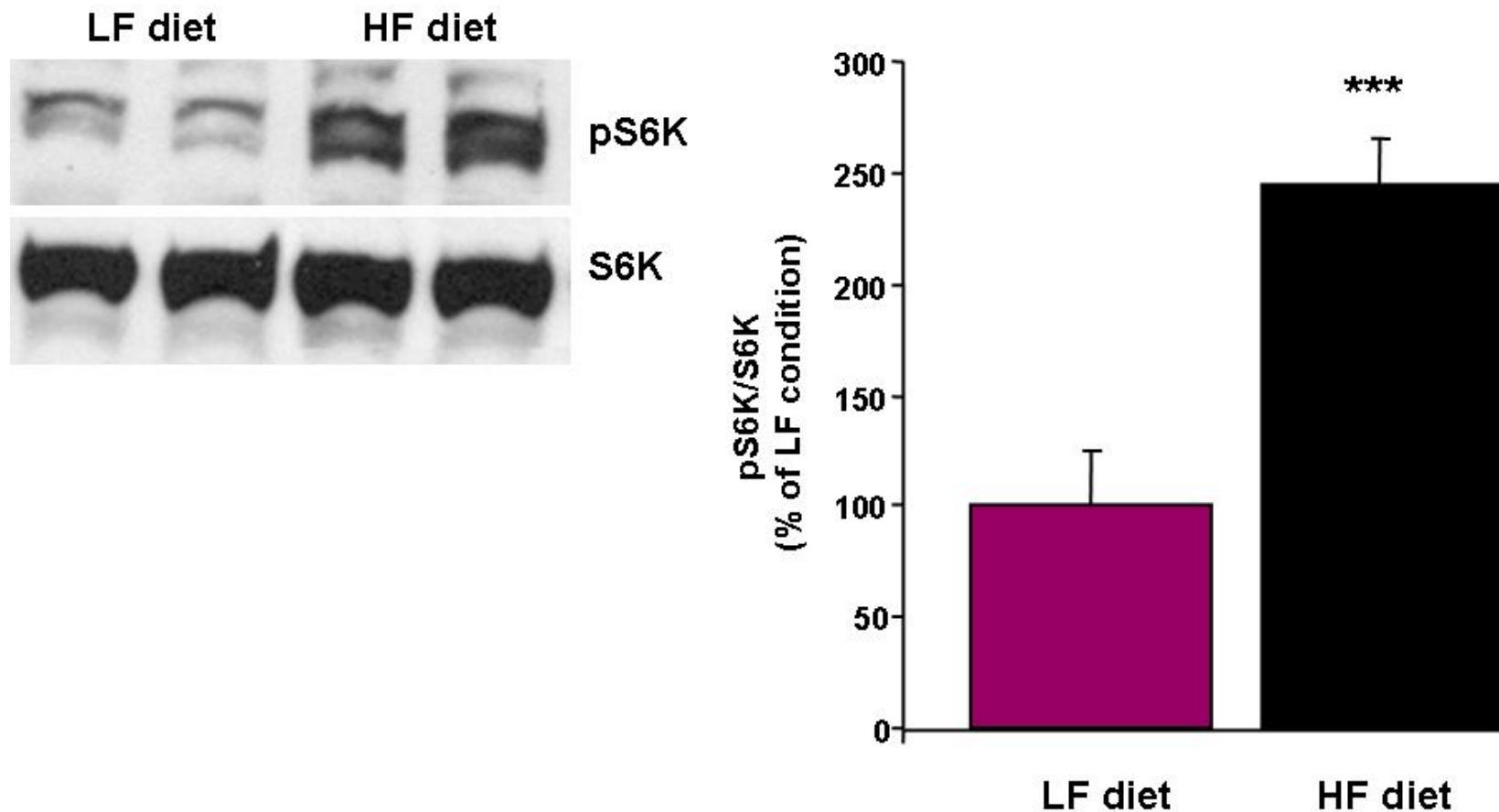
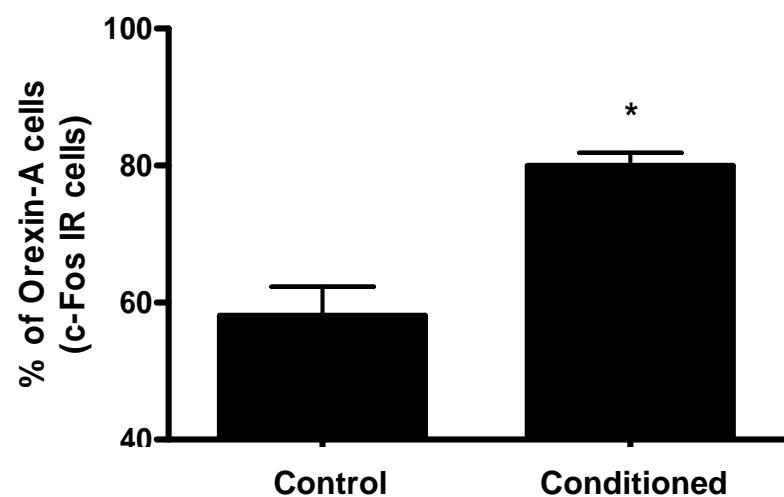
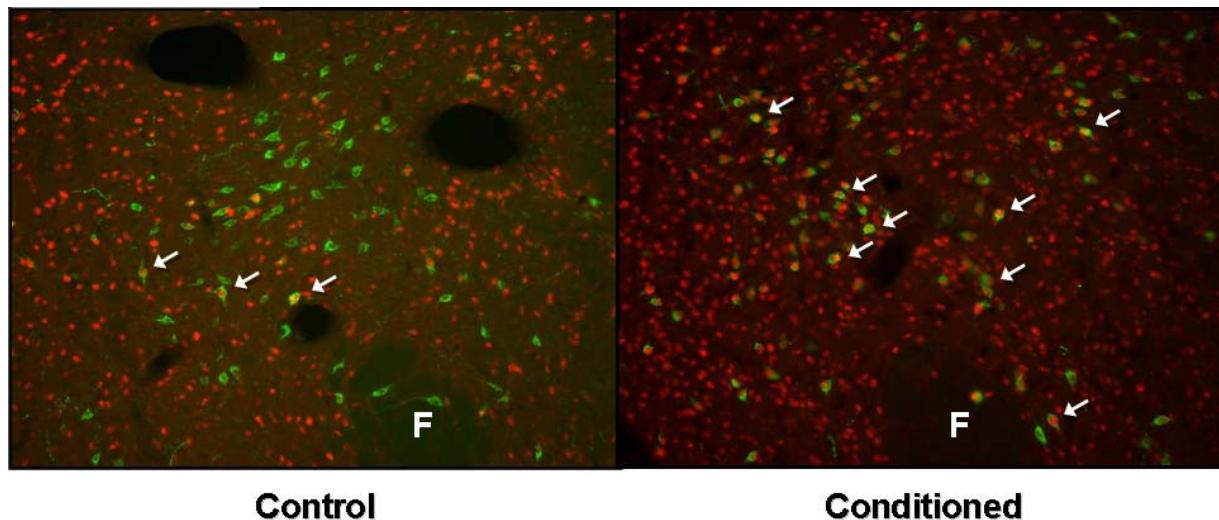


Figure 3.

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# Figure 4.

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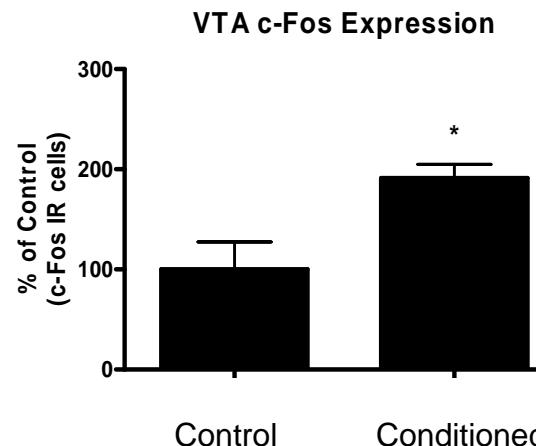
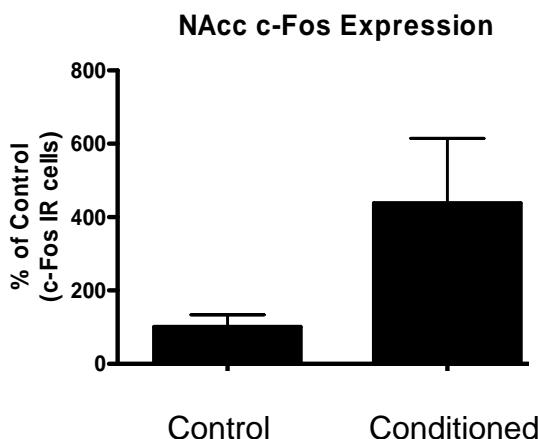
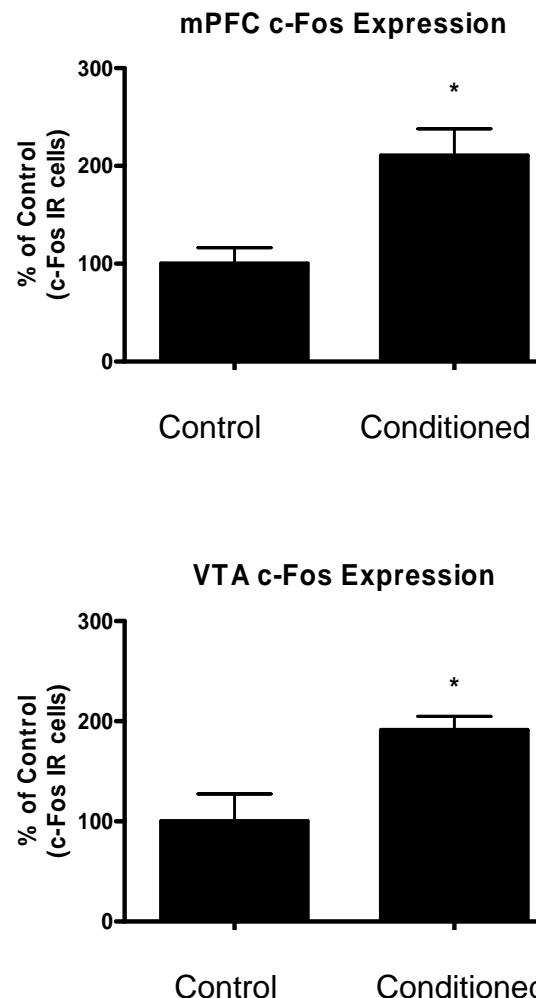
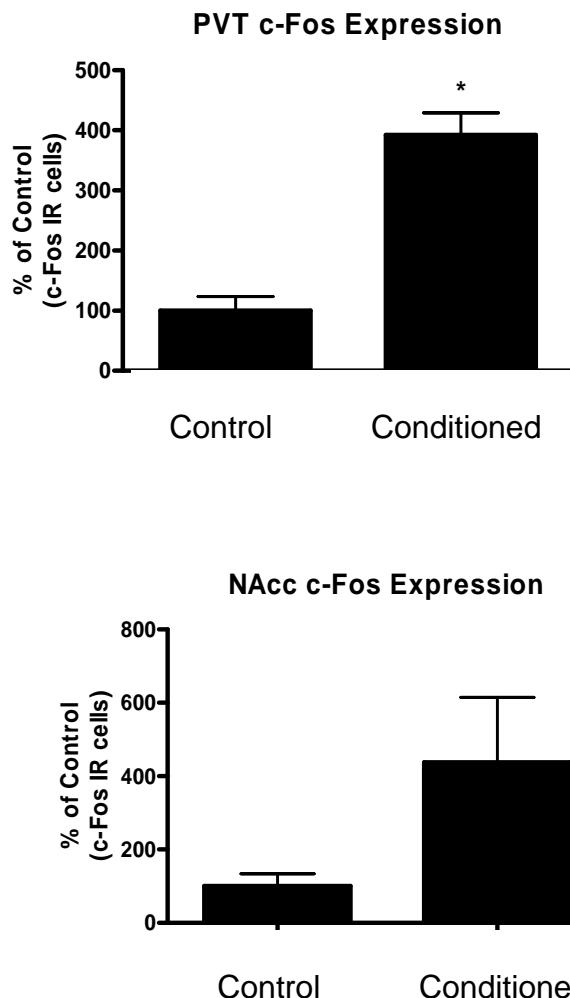
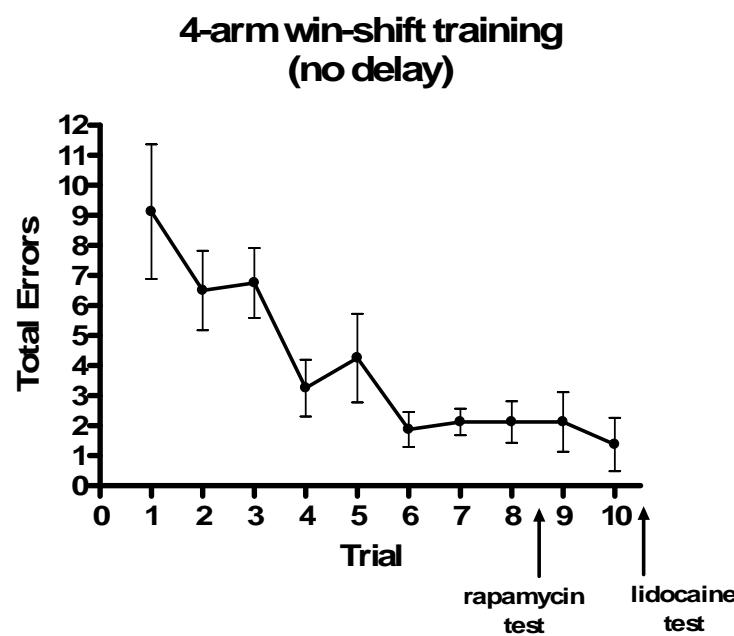
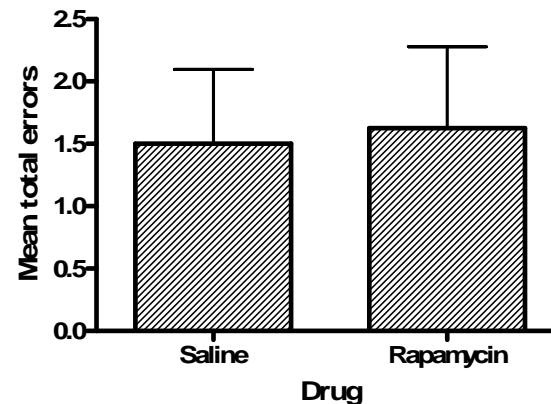


Figure 5.

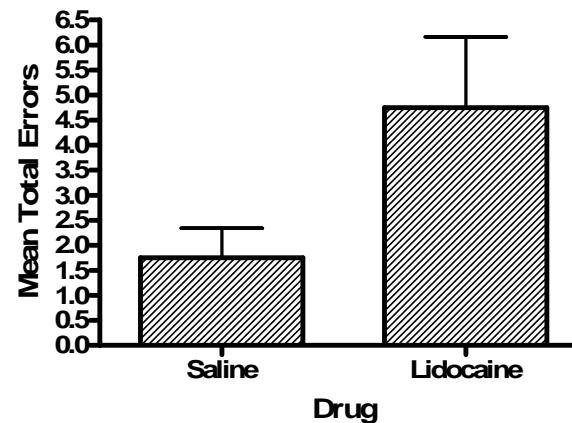
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**Rapamycin test  
(0.9 ng bilateral, 30 min prior)**



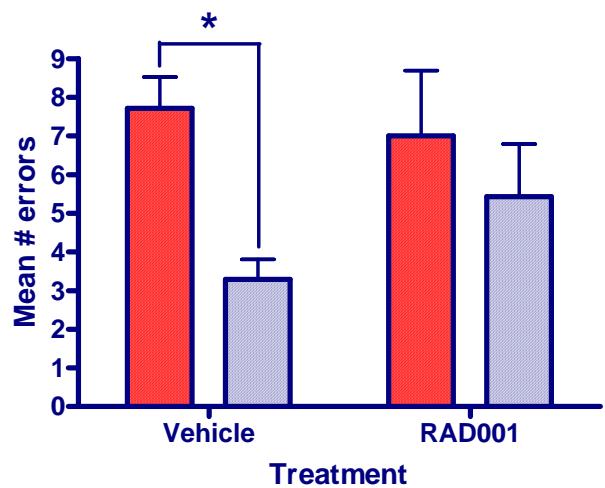
**Lidocaine test**



# Figure 6.

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## Reference memory errors



## Working memory errors

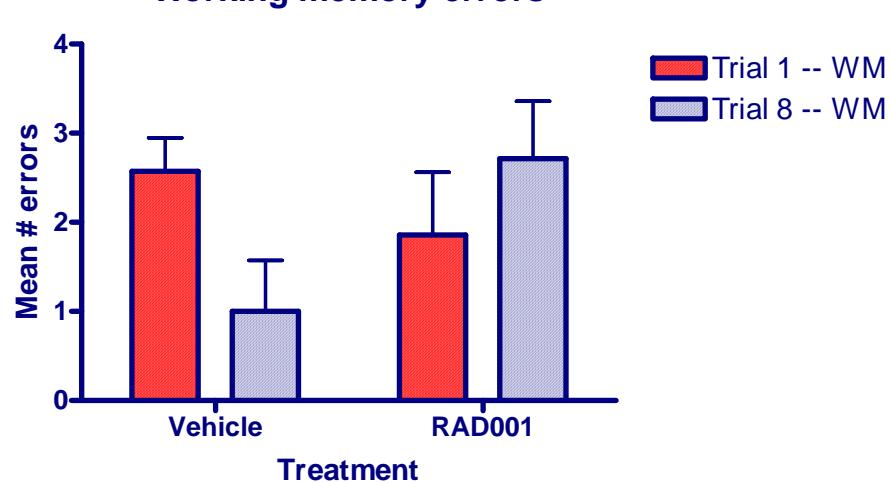


Figure 7.

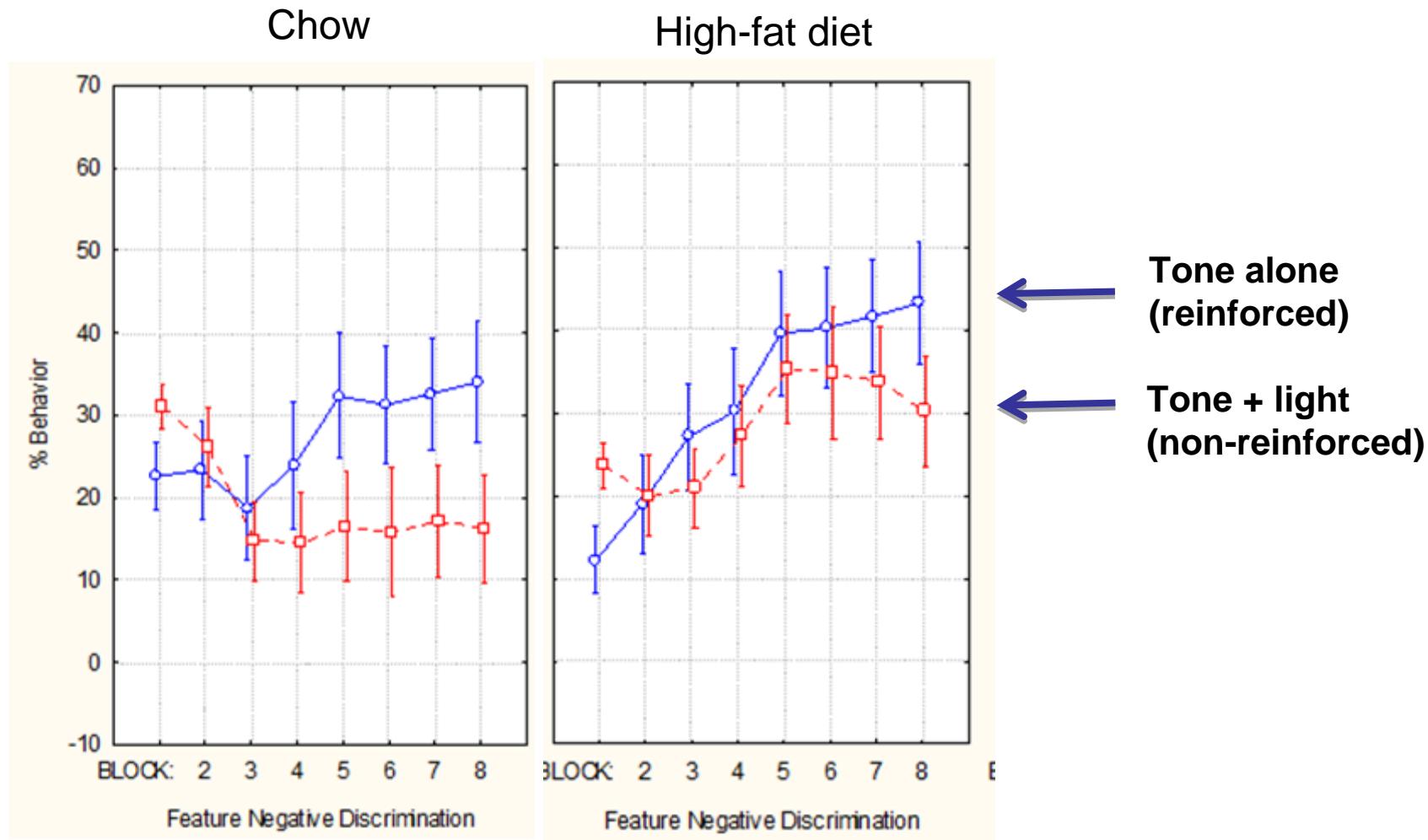


Figure 8.

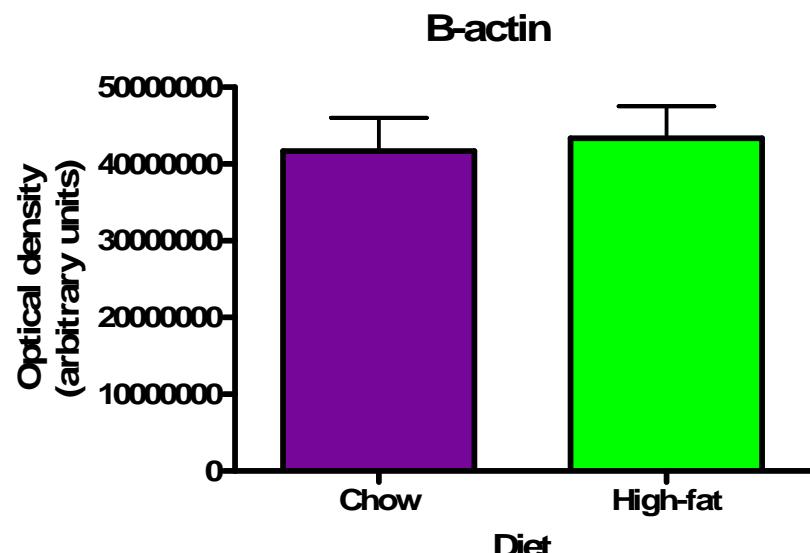
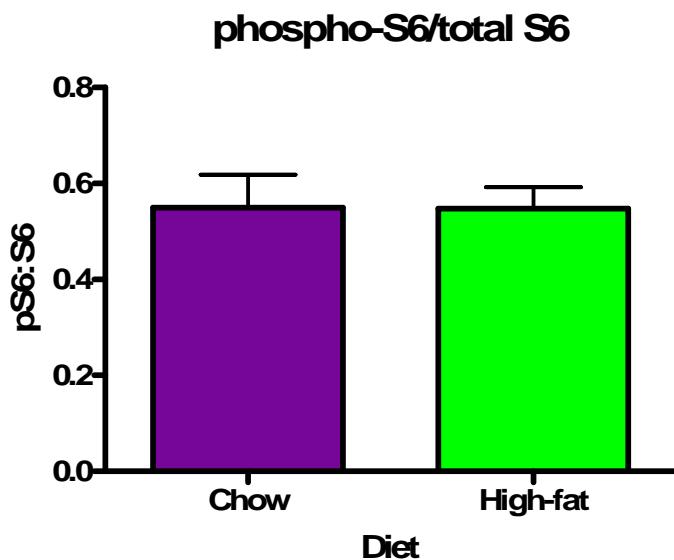
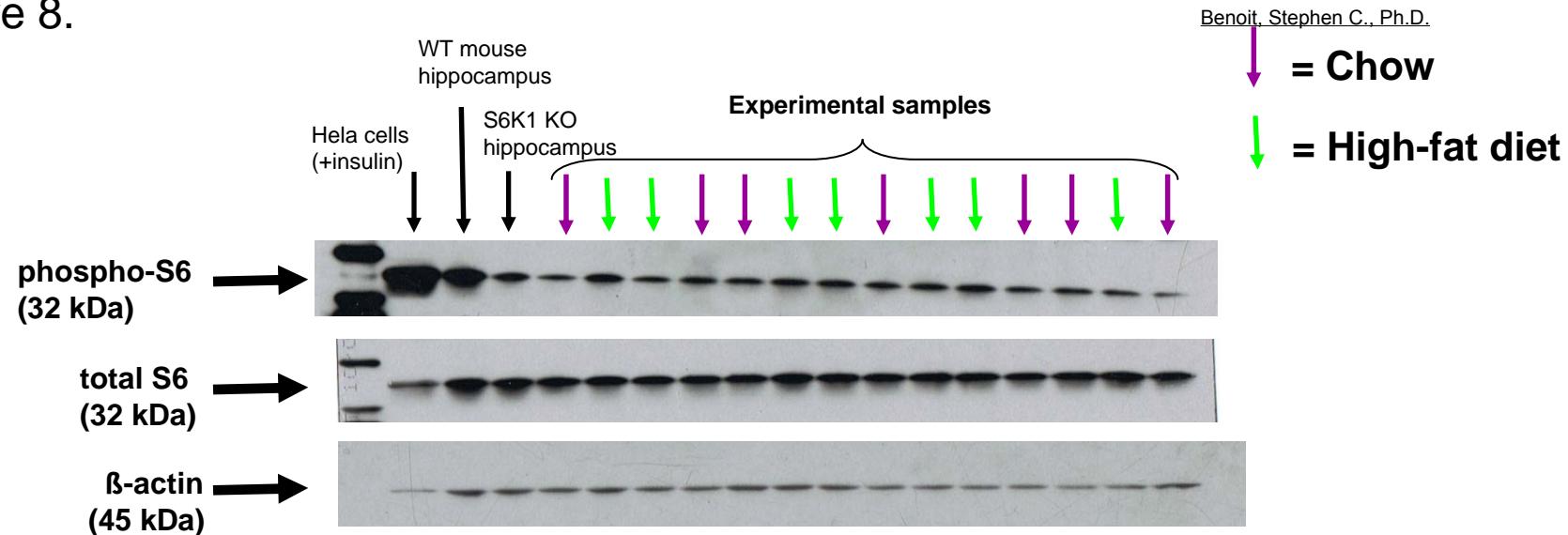


Figure 9.

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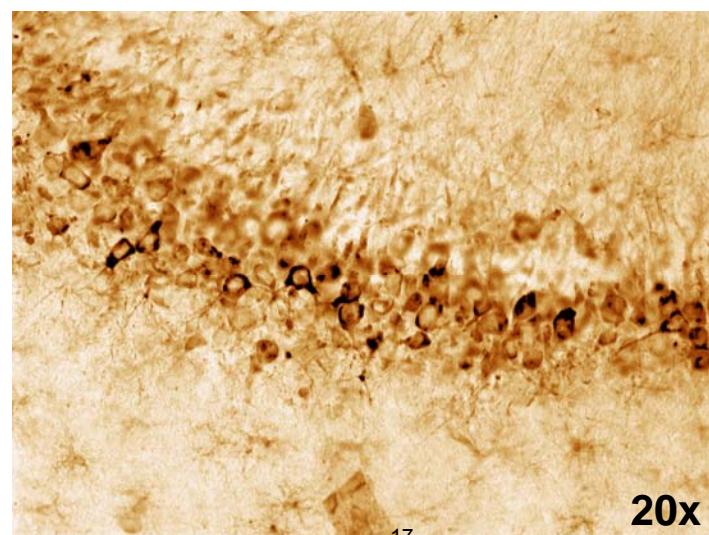
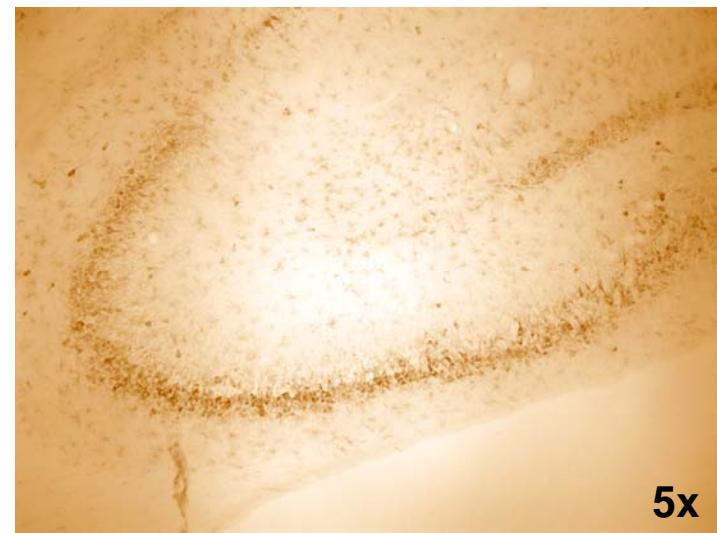
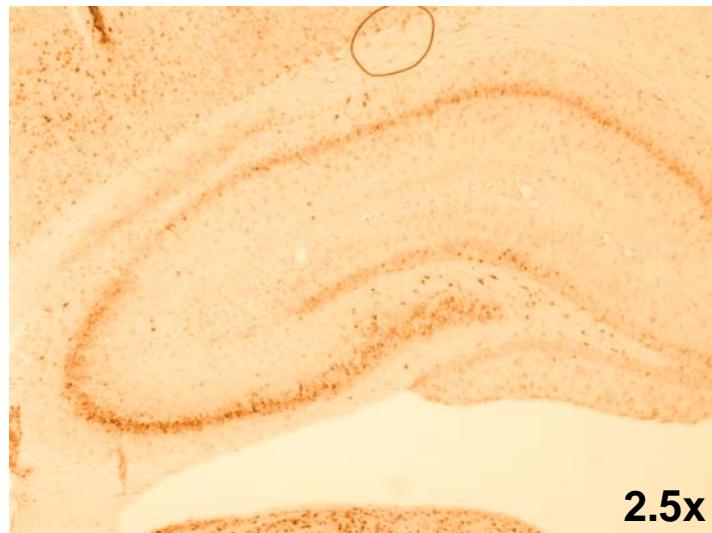
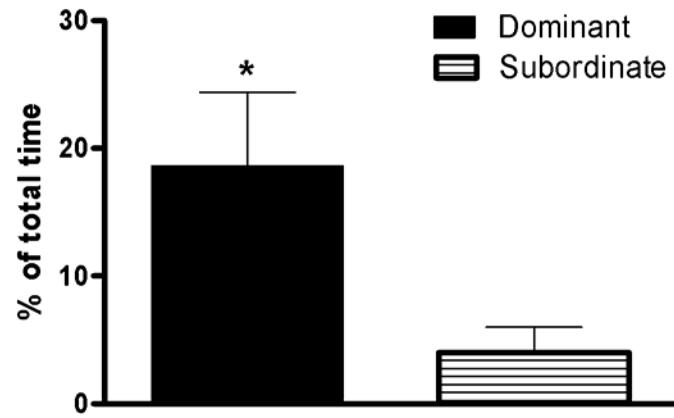


Figure 10.

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Time in Open Arms



Progressive Ratio Responding

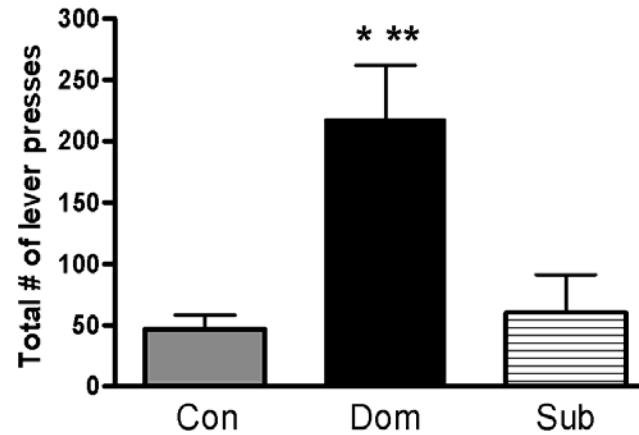
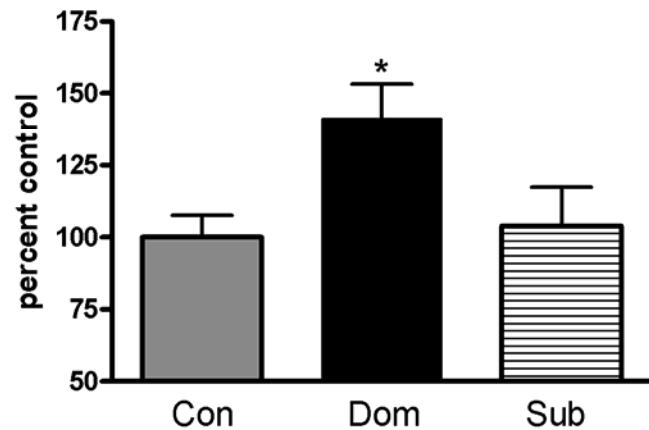


Figure 11.

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A

mPFC ORX-1R mRNA expression



B

mPFC ORX-2R mRNA expression

